THE DETECTION OF COMPLEMENT-FIXING ANTIBODIES FOR *RICKETTSIA RICKETTSII* IN THE SERUM OF *LEPUS CALIFORNICUS MELANOTIS*, MEARNS (BLACK-TAILED JACK RABBIT)

by

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INTRODUCTION

The etiological agent of Rocky Mountain spotted fever is Rickettsia rickettsii. The rickettsiae are transmitted among mammals by ticks.

The organism is present in numerous species of ticks. In the western mountain region the wood tick, Dermacentor andersoni, is involved; the dog tick, Dermacentor variabilis is found through the middle western and eastern states (Jellison, 1945); the rabbit tick, Haemaphysalis leporis-palustris is found widely distributed throughout the United States (Green et al., 1943).

In addition to man and the ticks themselves the organism is known to infect various species of rabbits, squirrels and mice. These animals are not considered to have a natural infection but serve as temporary passive carriers during the active life cycle of the tick. Mammalian blood apparently contains an "activator" substance which serves to stimulate the growth of the organism in the tick (Merchant and Packer, 1956). It is during this period of tick activity that man becomes infected.

There is evidence (Davis, 1953; Green et al., 1943; Lechleitner, 1959) that the spread of this infection from hares to humans is improbable by means of ticks, since the ticks present in the jack rabbits do not ordinarily parasitize man. However, the presence of Rocky Mountain spotted fever in the area is indicated and man could become infected from some other source (Lechleitner, 1959). Since the rickettsiae are present in all the tissues of the tick and in the feces, man may become infected by crushing the ticks

or by handling tick-infested animals (Merchant and Packer, 1956). Thus this represents another potential hazard to man if he is to handle the jack rabbit.

Several studies have been conducted with rabbits to determine if these animals may be a source of infection for man or domestic animals. The primary objective of these studies has been to determine if the ticks which parasitize this animal carry the rickettsiae of Rocky Mountain spotted fever, and if the sera of the rabbits contain antibodies against the causative agent.

Antibodies for the Rocky Mountain spotted fever organism may be found in rabbits infected with H. leporis-palustris, D. andersoni, D. variabilis, A. americanum, R. sanguineus, and perhaps other species of ticks (Lackman, 1960).

Since the black-tailed jack rabbit, Lepus californicus melanotis, Mearns, is numerous in the area of Lakin, Kansas, a study was undertaken to detect complement-fixing antibodies against Rickettsia rickettsii in the sera of these animals.

REVIEW OF LITERATURE

Rocky Mountain spotted fever has been known for many years in the Rocky Mountain region in states such as Idaho, Montana, Wyoming, Oregon and Washington. In 1930, however, it was found in the eastern and south atlantic states such as Maryland, Virginia, West Virginia, and North Carolina. Although the disease is not limited to this region, of the 2190 cases reported from 1933 to 1937, 65.5 per cent were from the mountain and pacific states and 27.4 per cent

from the south atlantic group, the two areas combined accounted for 93 per cent of the total cases reported in the country (Burrows, 1956).

The disease was first described in 1910 in Montana (Davis, 1953). It is customary to speak of two types of the disease, an eastern type and a western type. Both are, however, immunologically identical and are caused by the same rickettsia, Rickettsia rickettsii.

The rickettsiae are transmitted from mammal to mammal by ticks. Many kinds of mammals and ticks are involved. In many cases the larval stages live on different hosts than do the adult stages.

The dog tick, D. variabilis, which is found throughout the middle western and eastern states, feeds on many kinds of mammals and is probably the chief means of human infection (Davis, 1953).

The rabbit tick, Haemaphysalis leporis-palustris, is found throughout the United States, north into Canada and Alaska, and south into South America (Merchant and Packer, 1956). The larval stages may spread far and wide. The ticks may transmit the rickettsiae to their young through the egg (Davis, 1953; Philip, 1959). Rabbit ticks feed exclusively upon rabbits and hence cannot transmit the disease to humans (Davis, 1953). There is only one report (Brown, 1945) of the rabbit tick, Haemaphysalis leporis-palustris, parasitizing man. The man who had previously handled some rabbits found a half-engorged tick attached to the inside of his left forearm just below the elbow. The tick was later identified by J. H. Brown as Haemaphysalis leporis-palustris Packard.

A possible mechanism which could enhance the starting of new lines of infection where susceptible rabbits and hares are more abundant involves widespread infection in the rabbit tick Haemaphysalis leporis-palustris (Parker et al., 1951) and in Dermacentor parumapertus (Philip et al., 1955).

Evidence suggests that species of animals such as the cottontailed rabbit Silvilagus nuttallii may be important in the epidemiology of Rocky Mountain spotted fever in the western United States, and possibly the reservoir or one of the reservoirs of the rickettsiae in nature (Jellison, 1945).

Green (1943) states that rabbits and game birds may act as a reservoir of the disease. Rabbits may not have the natural infection, but they would act as passive carriers of the rickettsiae to other mammals (Merchant and Packer, 1956).

The laboratory diagnosis of Rocky Mountain spotted fever in humans or animals is difficult if one attempts to isolate the rickettsiae from the patient or animals. This can be satisfactorily performed only if guinea pigs are inoculated immediately upon withdrawal of the blood (Plotz and Wertman, 1942). The results obtained in the investigations by Plotz and Wertman, 1942, indicate that the complement fixation test could be employed in the diagnosis of Rocky Mountain spotted fever. They examined the sera from nine cases of Rocky Mountain spotted fever and obtained positive complement fixation reactions in all. The oldest case (HU) had the disease 4 1/2 years prior to the examination of the serum, while the most recent serum (LO) examined was obtained on the twelfth day of illness. This

latter finding is significant for if in subsequent cases it can be shown that antibodies can be demonstrated so early in the disease the complement fixation test may prove to be a real aid in diagnosis. Using sera from infected guinea pigs they obtained fixation in eight of their sera.

In recent years the complement fixation test has been used in the detection of antibodies for Rickettsia rickettsii in rabbit sera. The presence of complement fixing antibodies may suggest that the animal had the particular infection or that it is merely a reservoir for the organism which is found in the tick.

During the years 1951 and 1952 studies were conducted in Nevada in a population of black-tailed jack rabbits (Philip et al., 1955). Serological evidence indicated that in both years there was some exposure of the jack rabbits to western equine encephalomyelitis, rickettsial spotted fever and brucellosis. These investigators found that 35 of 260 sera of Northern Nevada jack rabbits showed complement-fixing antibodies for Rocky Mountain spotted fever. A selected series of the hare sera obtained in 1952 that gave positive complement-fixing reactions against spotted fever antigen was subjected to toxin-neutralisation tests on mice by Bell (Philip et al., 1955). Four of eight were also positive by this test in serum dilutions between 1:8 and 1:64; the other four gave no neutralisation. It is of interest to note that of five others which were negative by complement fixation methods, two gave positive neutralisation titers of 1:16 and 1:32 respectively.

Studies on the black-tailed jack rabbit (Lechleitner, 1959) in the Sacramento Valley, showed antibodies for Rickettsia rickettsii

in the sera of the hares. Specific complement-fixing antibodies for Rocky Mountain spotted fever were present in 10 of the 142 sera tested. Complement fixing titers of 1:16 or greater were considered positive. It is of interest to note that there was a seasonal incidence of the positive sera obtained, since they all occurred at a time of the year when the ticks were fully engorged and were present in greater numbers on the hares. March, April, May, and September were the months during the years 1955 and 1956 when positive results were obtained.

In a recent study among the fauna of the Great Salt Lake Desert in Utah (Stoenner et al., 1959) it was shown that a high percentage of jack rabbits possessed antibodies against Rickettsia rickettsii. Of the 773 species of Lepus californicus sera tested for complement-fixing antibodies, 193 were found to be positive. The serological evidence of the high percentage of jack rabbits possessing complement-fixing antibodies against this organism is an index of the prevalence of the organism. Antibodies were also found in the kangaroo rats. These two mammals are major hosts of adult and immature stages of the tick Dermacentor marmarpertus, and presumably they play a significant role in maintenance of the disease in nature.

EXPERIMENTAL METHODS

Collection of Specimens

Field specimens were collected monthly from September, 1957 to May, 1960. Mr. Richard Bowen collected serum samples from September, 1957 to August, 1959.

The serum samples used in this study were obtained from jack rabbits collected in eastern Kearny County, Kansas. The jack rabbits were killed in the Arkansas River Valley within a 20 mile radius of Lakin in southwestern Kansas. The jack rabbits were taken between the hours of 3:00 p.m. and 12:00 midnight, sometimes as late as 3:00 a.m. by means of a .22 caliber rifle. A 6-volt spotlight was used to aid in collecting after sunset.

Blood samples for the serological examinations were taken by cardiac puncture immediately after the rabbit was killed. Usually the heart continued to beat for several minutes after the rabbit was shot. Therefore, it could be readily palpated on the left side of the sternum from the level of the third to sixth rib.

The blood was removed with a 10 ml syringe using a one and one-half inch, 18-20 gauge needle. The blood obtained, usually 10 ml., was transferred to a sterile screwcap test tube and allowed to clot. After clotting, the blood was rimmed with a wooden applicator stick and placed in an ice chest overnight. After centrifugation the serum was removed from the test tube and returned to the laboratory in individually marked sterile vials for examination. The serum samples were stored in an ice chest for 24-48 hours while in the field and placed in the freezing compartment of a refrigerator for storage at -18° C in the laboratory. The sera were tested for complement-fixing antibodies against Rickettsia rickettsii.

Serological Examination

Preparation of Antigen: Two antigens were used during the course of this investigation. The antigens used in testing the sera were kindly supplied by Dr. David B. Lackman, from Rocky Mountain Laboratory, Hamilton, Montana. Spotted Fever antigen No. 672 was used in a dilution of 1:4 since it was found that it was anticomplementary in a dilution of 1:2. Spotted Fever antigen RLP 669 was used in a dilution of 1:8.

The rickettsial antigen was prepared according to the method of Topping and Shepard (1946). Fertile hens eggs were incubated 6 days and inoculated via the yolk sac with a dose of seed-yolk sac emulsion producing death in the majority of embryos in 8 - 10 days. Yolk sacs showing good growth of rickettsiae when suitably stained and examined microscopically, were harvested and stored in jars at -45° C. The day before the antigen was to be prepared, the jars were placed in the refrigerator at 6° C to permit thawing to proceed slowly. Yolk sacs were then emulsified in a Waring Blendor with sufficient Snyder's solution of saline to yield a 10 per cent suspension by weight. The use of Snyder's solution instead of physiological saline for emulsifying yolk sacs has sometimes yielded antigens with lower anticomplementary titers (Lackman, 1960). The pH was adjusted to 5.5-5.7. The emulsion was then extracted with one and one-half volumes of diethyl ether in a Squibb separatory funnel at 6° C and allowed to stand overnight. The emulsion separated into three distinct phases--a clear yellow at the top, an interphase of extraneous material containing some rickettsiae,

and the aqueous phase which, with some of the strains, contains most of the rickettsiae plus the soluble antigen released from the rickettsiae by exposure to the ether. It was desirable to separate the rickettsiae from the soluble antigen, and this was done by centrifugation of the aqueous phase. After the aqueous phase was removed the excess ether was taken off under vacuum. This constituted the antigen which was distributed in 3% of saccharin and stored at -15° C.

The undiluted antigen was kept frozen at a temperature of -15° C since it was found during the course of the study that the undiluted antigen when kept at refrigerator temperature (4-8° C) became unstable after a 2 months period.

Preparation of antisheep hemolysin. The hemolysin used in this study was obtained from the Colorado Serum Company, Denver, Colorado.

The hemolysin was obtained by giving rabbits repeated injections of a suspension of washed sheep red blood corpuscles every five days. The rabbits were then exsanguinated and the sera separated from the clot, pooled and preserved by the addition of an equal volume of neutral glycerin. A 1:100 dilution of hemolysin was then prepared for stock hemolysin by diluting 2 cc of glycerinated hemolysin with 98 cc of sterile 0.85 per cent salt solution. This 1:100 dilution was kept at refrigerator temperature for not more than 2 weeks at the end of which time new stock hemolysin was prepared.

Preparation of complement. Fresh pooled guinea pig sera preserved by rapid desiccation in vacuo from the frozen state was

used throughout the study. The lyophylized complement was obtained from the Colorado Serum Company, Denver, Colorado.

To restore the dehydrated complement to the original liquid condition 3 ml of restoring solution was added. The restoring solution was composed of 6 per cent sodium acetate in 2 per cent aqueous boric acid solution. The ampule was then gently shaken three to four minutes until the contents were completely dissolved. This procedure was followed during the examination of the first 500 sera. During the rest of the study this procedure was changed as follows: the ampule was shaken for 10 seconds and was set in the icebox overnight, at the end of which time the contents were completely dissolved. This second procedure accounted for less loss in titer of the restored complement.

Preparation of sheep red blood corpuscles. Blood was collected aseptically from the external jugular vein into a sterile container with sterile glass beads for defibrination by shaking. The blood was then kept at refrigerator temperature (4-5° C).

A 2 per cent suspension of sheep red blood corpuscles was prepared every day the test was to be run. Sheep red blood corpuscles were washed by adding sterile 0.85 per cent salt solution and centrifuging for 5 minutes at 2000 RPM. The supernatant fluid was then removed with a capillary pipette and the procedure repeated three times until the cells were packed evenly and firmly. For every 0.2 ml of packed cells the final volume was brought up to 10 ml with sterile 0.85 per cent salt solution, which represented a 2 per cent sheep red blood cell suspension. The sheep corpuscles employed in the test were obtained from blood not more than 7 days old.

Hemolysin titration.

1. The following dilutions were prepared from the 1:100 stock dilution of hemolysin.

No. 1 1.0 ml of a 1:100 dilution of hemolysin + 9.0 ml saline = 1:1000
No. 2 0.5 ml of a 1:1000 dilution of hemolysin + 0.5 ml saline = 1:2000
No. 3 0.5 ml of a 1:1000 dilution of hemolysin + 1.0 ml saline = 1:3000
No. 4 0.5 ml of a 1:1000 dilution of hemolysin + 1.5 ml saline = 1:4000
No. 5 0.5 ml of a 1:1000 dilution of hemolysin + 2.0 ml saline = 1:5000
No. 6 0.5 ml of a 1:3000 dilution of hemolysin + 0.5 ml saline = 1:6000
No. 7 0.5 ml of a 1:4000 dilution of hemolysin + 0.5 ml saline = 1:8000
No. 8 0.5 ml of a 1:5000 dilution of hemolysin + 0.5 ml saline = 1:10,000
No. 9 0.5 ml of a 1:6000 dilution of hemolysin + 0.5 ml saline = 1:12,000
No. 10 0.5 ml of a 1:8000 dilution of hemolysin + 0.5 ml saline = 1:16,000
No. 11 0.5 ml of a 1:8000 dilution of hemolysin + 1.0 ml saline = 1:12,000
No. 12 0.5 ml of a 1:10,000 dilution of hemolysin + 0.5 ml saline = 1:20,000

2. Two-tenths ml of these dilutions were transferred to tubes (7/8 x 11-12mm) and to each of these tubes was then added:

0.4 ml saline

0.2 ml complement diluted 1:40

0.2 ml sheep red blood corpuscles (2 per cent)

3. The tubes were shaken individually after each addition of a reagent and incubated in the 37° water bath one hour.

4. The unit of hemolysin was considered to be the highest dilution of hemolysin giving complete sparkling hemolysis. Two units of hemolysin were used in the test and therefore, the dilution of hemolysin to be used is that one which has twice the concentration of the unit.

Complement titration. The complement was titrated, using a 1:40 dilution prepared by combining 0.2 ml of complement and 7.8 ml of cold sterile 0.85 per cent salt solution.

A 0.2 ml pipette graduated in hundredths was used for measuring the following amounts of diluted complement: 0.05, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, and 0.22 ml. Sterile 0.85 per cent salt solution was added in such amounts that the volume in each tube was 0.4 ml. The antigen in the dilution used in the test was added in amounts of 0.2 ml. The tubes were then shaken individually after the addition of each reagent.

The complement mixtures were then incubated in a 37° C water bath one hour, after which 0.6 ml of sensitized sheep cells were added (equal parts of 2 per cent sheep cells and the dilution of hemolysin which contained 2 units per 0.2 ml. prepared by thorough mixing 10 minutes previously).

Readings were made after another hour of incubation at 37° C. The smallest amount of complement just giving complete spotting hemolysis was considered to be the exact unit. The next highest concentration was the full unit. In combining the antigen titration and complement fixation tests, two full units were employed and so diluted as to be contained in 0.2 ml.

Antigen titration. The antigen was titrated by preparing a 1:4 dilution of antigen by adding 0.3 ml of sterile 0.85 per cent saline to 0.1 ml of antigen. Twofold dilutions were then prepared ranging from 1:4 to 1:128. The twofold dilutions were made by carrying over 0.2 ml amounts of diluted antigen to 0.2 ml amounts

of saline contained in the tubes for the higher dilutions.

Once the antigen was titrated against a standard positive serum, one was chosen of a moderately high titer. This serum had a titer of 1:128. The serum was then diluted 1:64 to be used in the antigen titration. This was done by diluting 1 ml of 1:128 positive serum into 63 ml of sterile 0.85 per cent salt solution. Fortieths of ml was then transferred to the 0.2 ml amounts of diluted antigen contained in the tubes. To this mixture was added 0.2 ml of complement (2 units) in each tube.

After one hour incubation in the 37° C water bath the sensitized cells were added in 0.4 ml amounts. After further incubation in the 37° C water bath for one hour, the system was placed at the cold room temperature and read the following morning. The antigenic unit was considered to be the smallest amount of antigen giving a 4+ fixation.

TECHNIQUE OF THE TEST

For this study the method of Boughton (1944) was used for testing sera for complement-fixing antibodies against Histoplasma capsulatum.

Sera were inactivated at 56° C for one-half hour. Two-fold dilutions were used ranging from 1:4 to 1:512. The 1:4 dilution was prepared by adding 0.3 ml saline to 0.1 ml serum. Amounts of 0.2 ml were carried over to the 0.2 ml amounts of saline contained in the tubes for the higher dilutions. To the serum dilutions contained in the tubes were added 0.2 ml of the proper dilution of antigen (4 units) and 0.2 ml of complement (2 units).

After one hour incubation in the 37° C water bath the sensitized sheep cells were added in 0.4 ml amounts. After further incubation in the 37° C water bath for one hour, the tubes were placed at 4.8° C for 15-18 hours and then read. The amount of fixation was recorded as ++++ (complete), +++ (75 per cent), ++ (50 per cent), + (25 per cent), ± (trace), and 0 (none). The titer was recorded as the highest dilution showing ++++ or +++ fixation. The following controls were included.

Serum controls: Sera to be tested were diluted 1:4 and twofold dilutions made ranging from 1:8 to 1:32. To these serum dilutions were added the same reagents as used in the test except that salt solution was substituted for antigen. The purpose of this serum control was to check whether the sera under test was anticomplementary.

Antigen control: The antigen control contained twice the volume of the dilution of antigen used in the test, i.e., 0.4 ml and 0.2 ml of complement and 0.4 ml of sensitized cells. The purpose of this control was to check if the antigen was anticomplementary.

Hemolytic system control: The hemolytic system control consisted of four tubes containing 0.05, 0.1, 0.15, 0.2 ml of the dilution of complement used in the test, these amounts representing 1/2, 1, 1 1/2, and 2 units. The volume in each tube was made up to 0.6 ml with sterile saline, and 0.4 ml sensitized cells were added. The tube containing 0.05 showed 1 or 2+ fixation, and the three remaining tubes were completely hemolyzed.

Standard serum: A standard serum composed of a pooled lot of serums from recovered guinea pigs was titrated in each test using the same dilutions as for the serum under test. The purpose of this control was to show the sensitivity of the antigen.

Negative serum control: Sheep blood serum was titrated in each test, using the same dilutions as for the serum under test. The purpose of this control was to show the specificity of the antigen.

EXPERIMENTAL RESULTS AND DISCUSSION

Five hundred forty-nine serum samples from blood-bled jack rabbits were collected during the years 1957, 1958, 1959 and 1960 (Table 1). These sera were tested for complement-fixing antibodies against Histoplasma capsulatum. Those sera found to have a titer of

Table 1. Summary of specimens tested during the period (Sept. 1957-May 1960).

Number of sera tested							
1957		1958		1959		1960	
September	24	January	7	January	17	January	2
October	13	February	15	February	24	February	15
November	15	March	14	March	16	March	0
December	9	April	29	April	15	April	10
		May	27	May	17	May	15
		June	22	June	9		
		July	26	July	13		
		August	24	August	9		
		September	21	September	23		
		October	23	October	22		
		November	22	November	1		
		December	23	December	22		
Subtotal	61		260		185		43
Total number of sera tested							549

1:4 or greater by our method were sent to Dr. David H. Lackman, Rocky Mountain Laboratory and were tested by him for antibodies for Rocky Mountain spotted fever. Specific indication of complement-fixing antibodies was present in 22 samples of sera sent to Lackman. A summary of these results is found in Table 2. According to Lackman's method a titer of 1:16 or greater is significantly positive. Higher serum titers were obtained by Lackman.

The method used by Lackman for titrating antigens and serums by complement fixation was the one proposed by Kolmer, Pearson, and Mayer, 1935. Unit volumes of 0.2 ml are employed and 4, 50 per cent units of complement are used. This procedure apparently is more sensitive since he obtained higher serum titers on the positive sera we sent him. Perhaps the reason why higher serum titers are obtained by Lackman is that magnesium ions are added to the system. $MgCl_2$ is added to the veronal buffer which is used in the dilution of complement, sheep red-blood cells, hemolysin, antigen, and serum. Studies by Mayer et al., 1946, indicated that the presence and concentration of Mg^{++} ions were important in the hemolytic activity of guinea pig complement. They reported that insufficient magnesium is present in hemolytic tests as ordinarily performed, but addition of only 0.005 per cent $MgCl_2$ almost doubles the complement titers. Lackman also uses a 50 per cent hemolysis as the endpoint in titrations. Greater precision is obtained with a 50 per cent endpoint because the curve of hemolysis is steeper in this region (Wadsworth, 1947). In addition the positive sera were tested by Lackman for antibodies against typhus. No antibodies were found

against typing in the positive sera, thus this indicates that the antigen was serually specific for antibodies against Histoplasma capsulatum.

The 26 samples from jack rabbit sera which were found to be significantly positive represents 4.6 per cent of the total number of sera tested. Similar positive results for antibodies against Histoplasma capsulatum have also been obtained by other investigators.

Table 2. Results of the sera showing specific complement-fixing antibodies for Histoplasma capsulatum.

Serum Number	Date Collected	Titer	Titer*
833	September 24, 1957	1:32	1:128
880	November 22, 1957	1:8	1:64
931	November 23, 1957	1:16	
1026	February 27, 1958	1:8	1:40
1030	March 30, 1958	1:4	1:20
1063	April 25, 1958	1:12	1:48
1087	April 28, 1958	1:4	1:32
1094	April 28, 1958	1:8	1:32
1093	April 24, 1958	1:4	1:16
1103	June 14, 1958	1:16	1:64
1158	July 11, 1958	1:8	1:40
1215	July 12, 1958	1:16	
1266	July 12, 1958	1:8	1:32
1327	August 6, 1958	1:16	1:96
1439	September 20, 1958	1:4	1:24
1532	December 19, 1958	1:32	1:96
1587	December 19, 1958	1:16	
1758	April 17, 1959	1:4	1:24
1960	September 25, 1959	1:8	1:24
1994	October 22, 1959	1:8	1:24
2013	October 23, 1959	1:8	1:24
2080	December 12, 1959	1:8	1:24
2186	April 9, 1960	1:8	1:40
2211	May 5, 1960	1:16	1:40
2217	May 5, 1960	1:16	
2218	May 5, 1960	1:8	1:40

*Titer obtained by Dr. David T. Beckman using the method proposed by Culer.

Philip et al., 1955, found that 35 of 240 sera of Northern Nevada jack rabbits showed complement-fixing antibodies for Rocky Mountain spotted fever. Lechleitner (1959) found specific complement-fixing antibodies for the Rocky Mountain spotted fever organism in 9 of 142 sera tested. Stoenner et al., 1959, in studies done in the Dugway of the Great Salt Lake Desert in Utah showed that a high percentage of jack rabbits possessed antibodies against Pasteurella tularensis. All these studies made use of the complement fixation test to detect these antibodies and titers of 1:16 or greater were considered positive.

It is of interest to note that in this study the greatest number of positive samples was found during the months in which the ticks were fully engorged on the hares, that is, during the months from April to September, although some positive sera were also found during October, November and December (Table 3). The greatest number of positive sera was found during the month following peak infestation with ticks, that is, April, May, July and September. Similar results were obtained by Lechleitner (1959). He found that the greatest number of positives appeared during the months of March, April and September.

The presence of complement-fixing antibodies in the jack rabbits does not necessarily imply the animals are infected with the disease (Merchant, 1960; Lechleitner, 1959). The problem here is determining what we mean by the word infection. In many instances the mere presence of antibodies in the circulation is evidence of infection. It is safer to assume that infection is one in which the

Table 3. Rocky Mountain spotted fever complement-fixing antibodies in jack rabbit sera.

Date collected	Number of jackrabbits tested	Positive	Titer of positive
1957			
September	28	1	1:32
October	13	0	
November	15	2	1:8
			1:16
December	7	0	
1958			
January	7	0	
February	15	1	1:8
March	15	1	1:8
April	29	4	1:32
			1:4
			1:8
			1:4
May	27	0	
June	22	1	1:16
July	26	3	1:8
			1:16
			1:8
August	24	1	1:16
September	21	1	1:4
October	23	0	
November	22	0	
December	23	2	1:32
			1:16
1959			
January	17	0	
February	21	0	
March	15	0	
April	14	1	1:4
May	17	0	
June	9	0	
July	13	0	
August	9	0	
September	22	1	1:8
October	22	2	1:8
			1:8
November	1	0	
December	22	1	1:4
1960			
January	2	0	
February	15	0	
March	0	0	
April	10	1	1:8
May	16	3	1:16
			1:16
			1:8

presence of a parasite is evidenced by symptoms or lesions characteristic of the disease (Wenchant, 1960). For this reason we cannot say that the jack rabbits are infected with the disease but that they are merely accidental hosts of the organism.

Since the complement-fixing antibodies are present in the sera of these jack rabbits we can safely assume that the rabbit ticks which feed upon these animals are infected with the rickettsias. This is of extreme importance since it represents a potential hazard for man if he is to handle the jack rabbits. Man can easily become infected by crushing the ticks when handling the animals, since the rickettsias are present in all tissues of the tick and in the feces.

Although the rabbit tick Haemaphysalis leporis-palustris does not ordinarily parasitize man, the presence of antibodies against Dermacentor rickettsii is of significance since this indicates the presence of Rocky Mountain spotted fever in the area and man could become infected from some other source (Lackman, 1959).

SUMMARY

During the period (September 1957 to May 1960) black-tailed jack rabbits were collected in the area of Lehi, Texas. Serological studies were done on the sera of these animals to detect complement-fixing antibodies against Dermacentor rickettsii. Serological examination using the complement fixation test revealed the presence of specific complement-fixing antibodies for Rocky Mountain spotted fever in 26 of the 59 sera tested. Those sera having a titer of 1:4 or greater were considered positive by our method. These sera were sent to Dr. David H. Lackman from Rocky Mountain Laboratory who

confirmed the results. In addition the positive sera were tested for typhus antibodies and negative results obtained.

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THE DETECTION OF COMPLEMENT-FIXING ANTIBODIES FOR *RICKETTSIA RICKETTSII* IN THE SERUM OF *LEPUS CALIFORNICUS MELANOTIS*, MEARNS (BLACK-TAILED JACK RABBIT)

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The etiological agent of Rocky Mountain spotted fever is Rickettsia rickettsii. The organism is present in numerous species of ticks. Haemaphysalis leporis-palustris, D. andersoni, D. variabilis, A. americanum, D. sanguineus are species of ticks found parasitizing rabbits. Antibodies for the Rocky Mountain spotted fever organism may be found in rabbits infected with these species.

Since the black-tailed jack rabbit, Lepus californicus melanotis, Mearns, is numerous in western Kansas, a study was undertaken to detect the presence of complement-fixing antibodies against Rickettsia rickettsii in the sera of these animals.

Serological studies using the complement fixation test were made on the sera of 549 jack rabbits. Specific Rocky Mountain spotted fever antibodies were found in 26 of the 549 jack rabbit sera.

The presence of complement-fixing antibodies in the sera of these black-tailed jack rabbits does not necessarily imply that the animals are naturally infected. These animals serve as accidental hosts of the organism.

Although the rabbit tick Haemaphysalis leporis-palustris does not ordinarily parasitize man, the presence of the complement-fixing antibodies in the sera of these jack rabbits indicates that Rocky Mountain spotted fever is present in the sera and man could become infected from some other source.